U-dots for unique security tagging

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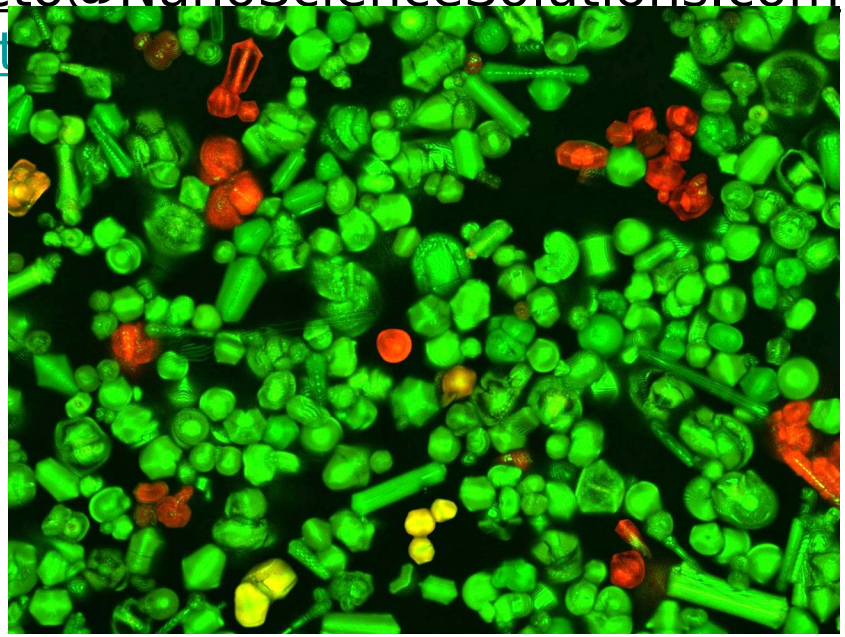
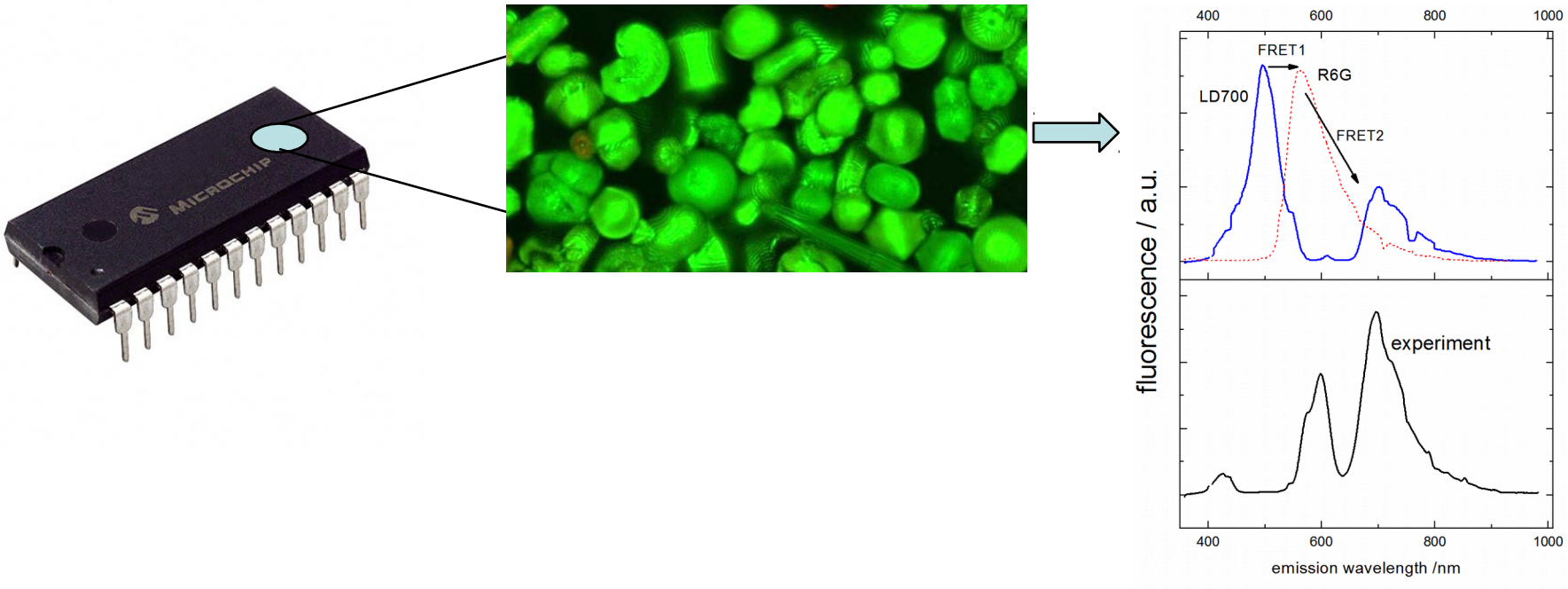


Idea: fluorescent labeling

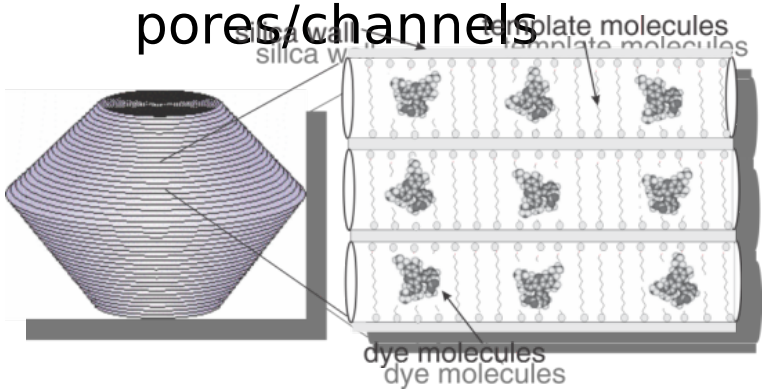
Labeling with special fluorescent particles: U-dots®

Complex fluorescent spectra that do not exist naturally



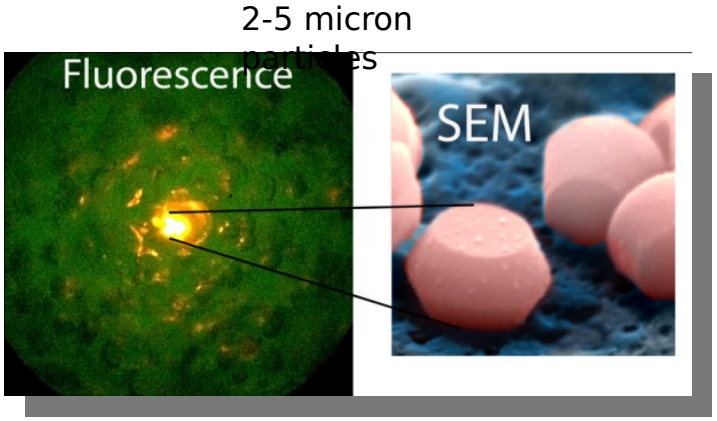
U-dots: Technology

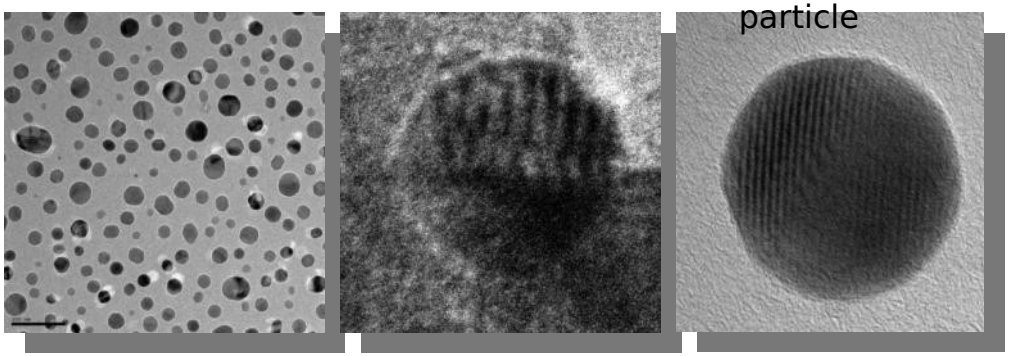
U-dots are silica nanoporous particles in which existing (including commercial) fluorescence dyes are encapsulated inside the pores/channels



U-dots can be

Micron-nano size



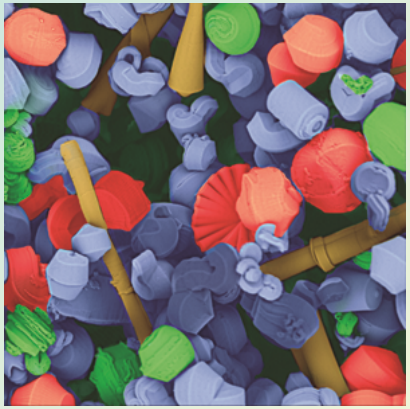


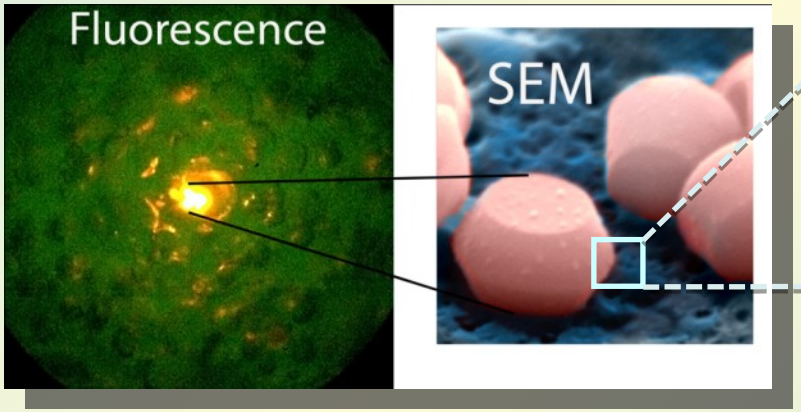
U-dots sizes can be between 8 nm and tens of microns

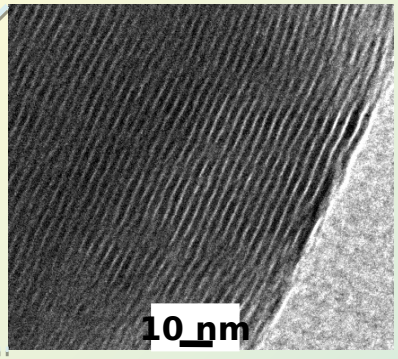
U-dots: Technology

All have cylindrical pores of nanosize diameter:

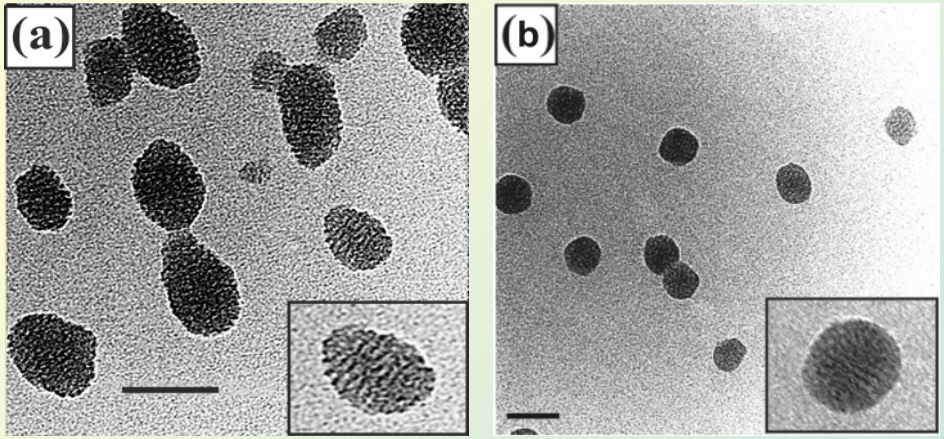
Micron particles







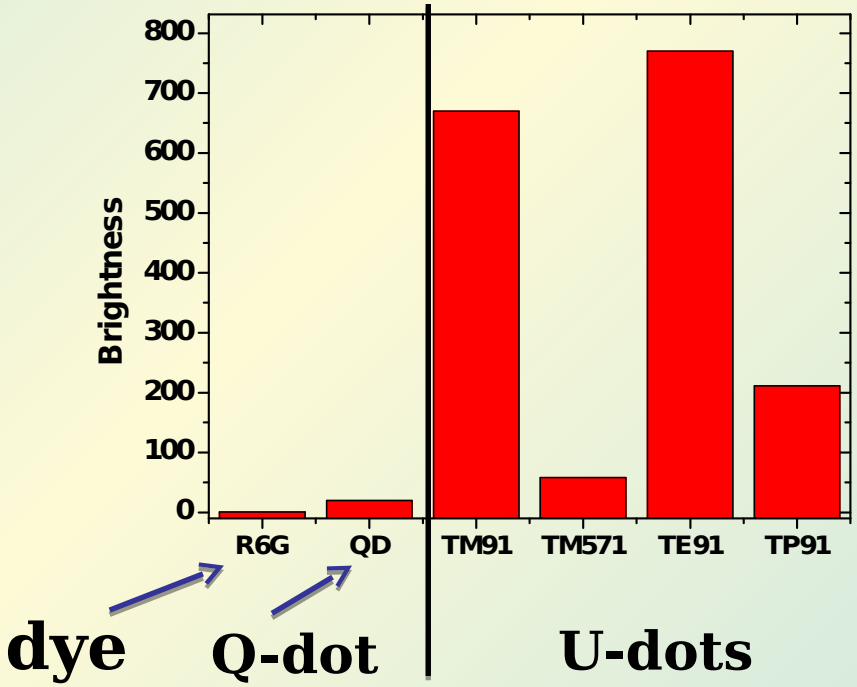
Nano particles



U-dots: Brightness

Brightness of 40 nm particles relative to 1 molecule of R6G dye and quantum dots (CdSe/ZnS green)

$$\text{FMSNP } relative brightness = \frac{FL_{\text{FMSNP}} / C_{\text{FMSNP}}}{FL_{\text{R6G}} / C_{\text{R6G}}} = \frac{brightness\ of\ one\ U\text{-}dots}{brightness\ of\ one\ dye\ molecule}$$



U-dots

Comparison with Q-dots and other fluorescent

	U-dots	Q-dots	Other particles
Size	8nm-10,000nm	5-60nm (water)	6nm-1000nm
Photostability	Relatively stable	**Highly stable**	Relatively stable
Thermal stability	Depends on dye	Depends some coating	Depends on dye
Ex. spectrum	Like dye (narrow) **Broad if FRET**	**Broad**, increasing towards UV	Like dye (narrow)
Em. spectrum	Like dye (broad)	**Narrower than dye**	Like dye (broad)
Single-molecule analysis	**very good, No blinking**	Good; limited by blinking	Unknown
Multiplexing	**Very high**	Up to 5 colors demonstrated	Limited
Toxicity	Expected none	Potentially high	**No**
Brightness	High (up to green) **Very high** (for green to NIR)	High	High to very high
Spectral broadness	**Very high**	**Very high**	Low

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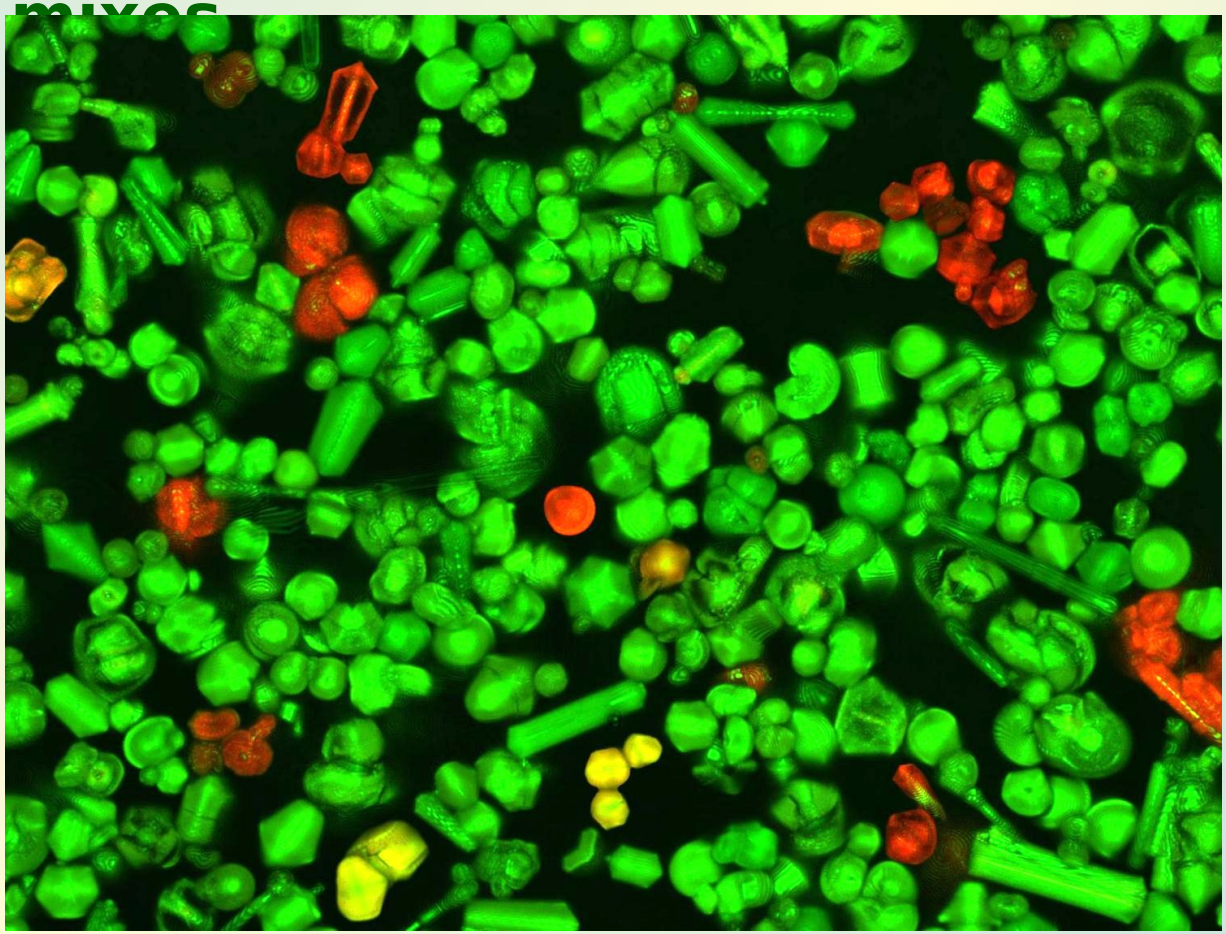
U-dots for color encoding

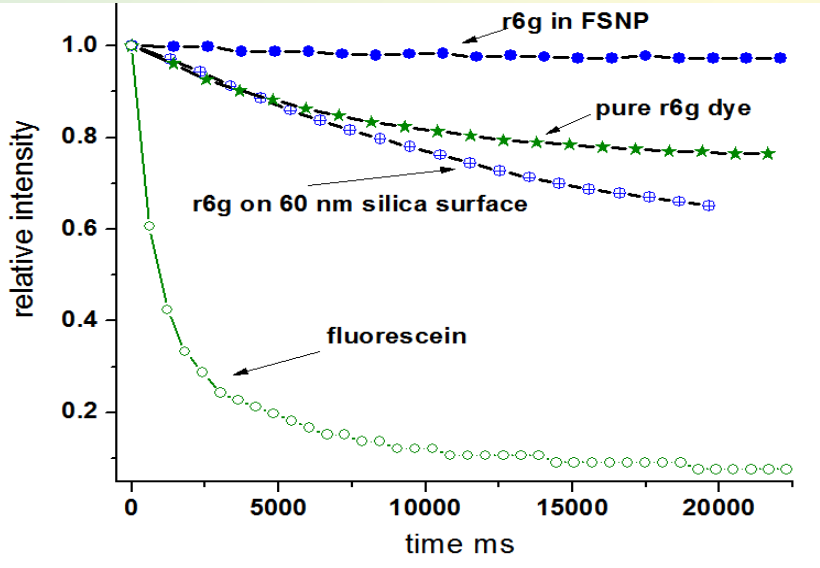
Example of fluorescence of micron U-dots containing various dyes and their mixes



Physical mix of 4 different dye compositions

U-dots: high stability

Photobleaching



A relative decrease of brightness of different fluorescent substances compared to fluorescent nanoporous silica nanoparticles (FSNP).

25 mW 488 nm laser in a scanning confocal microscope was utilized.

Long-term stability without intensive photobleaching:

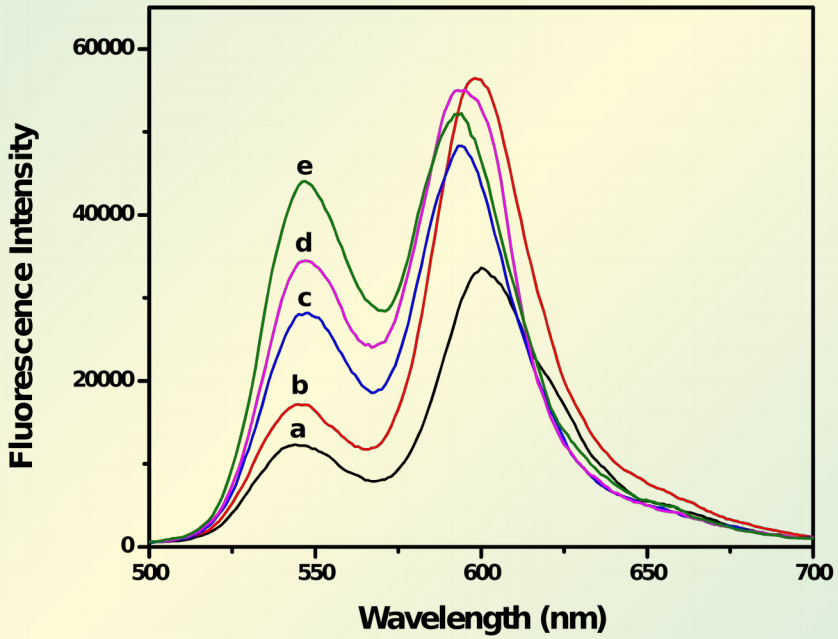
So far the spectral stability of R6G dye encapsulated in micron-size U-dots was tested evaluated. It was stable after 7 years of storage in ambient conditions in water. It is expected to be save for much longer in air or encapsulat

How many different combinations?

The total number comes from MULTIPLICATION of number from the following 3 categories:

1. Dyes with different spectra (~200) and their combinations:
   assuming 4 dyes: ~**65,000,000**
   assuming 3 dyes: ~**1,300,000**
   assuming 2 dyes: ~**20,000**
2. Different relative concentrations of dyes (~**5-10** for 2 dyes, **25-100** for 3 dyes, **125-1000** for 4 dyes).
3. Different spectra at different excitation wavelengths (~**5-10**)

Example of Different relative concentrations of dyes



Fluorescence spectra of particles encapsulating two fluorescent dyes at molar ratios of
a)10 b)20 c)50 d)70 e)90

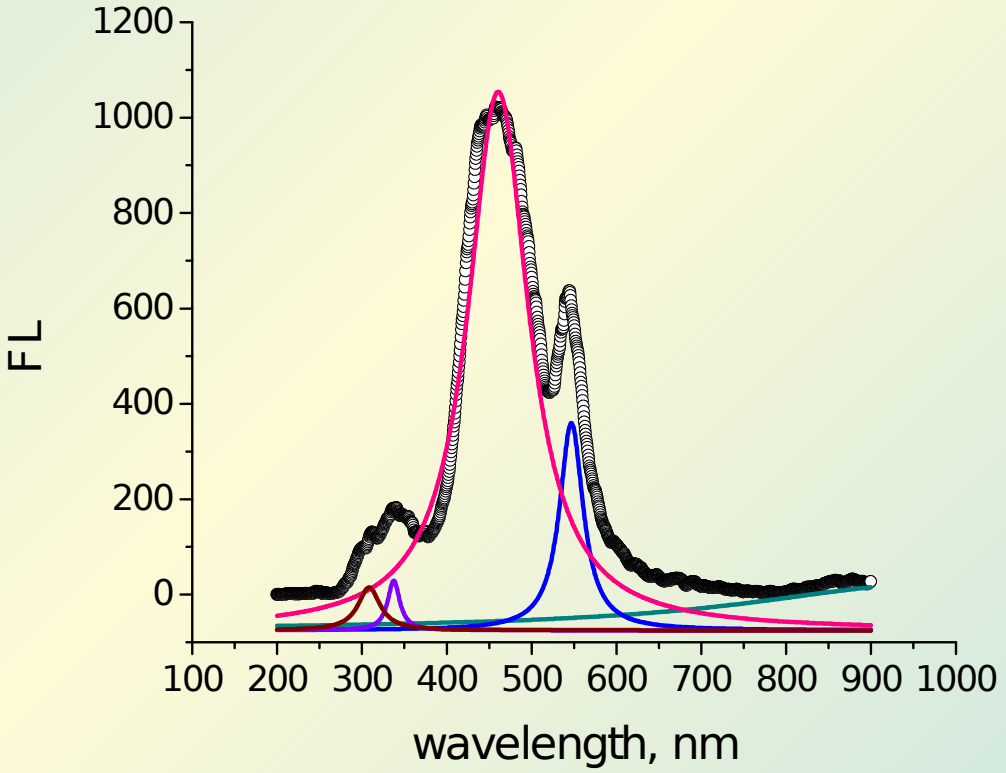
Example of spectral reading

Algorithm

$$I_{total} = \sum_{n=1}^{N} f_n I_n(l)$$

$$M = \int w \left| I(w) - \sum_{n=1}^{N} f_n I_n(w) \right|^2$$

$$\partial M / \partial f_{n=1,2,K,N} = 0$$



Unambiguous solution if spectra $I_n(w)$ are sufficiently different in the ***entire*** spectra range (the determinant of the Gaussian matrix of the linear equation is not equal to zero

The particles with the entirely overlapped spectra can still be reliably resolved.

Technology readiness and unsolved problems

The technology for U-dots is ready. NNS holds the exclusive license from Clarkson University for ultrabright fluorescent particles. Dr. Sokolov developed this technology while in the Department of Physics and Chemical and Biomolecular Sciences with a partial support from the US Army research office.

The problems still to be answered:

- Packaging of U-dots for security labeling applications.
- Spectral stability of packaged U-dots (though expected to be high) has to be studied.
- Incorporation of multiple dyes: non-linear effects of the concentration are to be investigated. This may add more multiplexing but it could be more subject to spectral change with time..

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Patents:

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- Igor Sokolov, Eun-Bum Cho, Dmytro Volkov “Syntheses of ultrabright fluorescent silica particles”, pending March 10, 2010
- I. Sokolov, S. Naik, “Syntheses of Ultra-bright Fluorescent Silica Particles”, full patent application filed 2007